Porous Materials of Hydroxyapatite/biopolymers Nanocomposites through Self-organization

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Abstract

With the advent of new treatment of tissue repairs, tissue engineering is one of the key technologies. There are three important factors; scaffold, mechanical stimulation and biochemical factor. In this study, we have developed porous materials of hydroxyapatite, polysaccharides and collagens nanocomposites as a novel scaffold in which the nanostructures were controlled through self-organization processes. The porous materials of the nanocomposites have the pore diameter of 200-300μm and a good biocompatibility to human chondrocytes.

Introduction

Tissues, organs and matrix in human bodies that constructed with inorganic (mainly calcium phosphate) and/or organic substances (mainly collagen) have highly ordered hierarchical structures at molecular levels. These organized structures show many intelligential functions for the lives to survive on the earth. For example, pro-collagens with *N* and *C*- terminus produced in endoplasmic of the cells are released at the outer fields and the pro-collagens are spontaneously assembled and formed the collagen fibrils with periodical structure of 67nm; the details of self-organization processes are more complex manners. In the macro region of bone, the hydroxyapatite (HAp) and collagen fibrils are highly ordered structures and bone shows strong mechanical and other specific properties. To architect the similar structures of our tissues, the formation processes of interfacial interaction governed by the competition and harmony of ionic, covalent, hydrogen and other weak bonds should be understood and controlled. In this study, we aimed to create the novel porous materials with hydroxyapatite and biopolymers (polysaccharides and collagens) under self-organization processes and examine the cell reaction properties using human chondrocytes.

Experiments

Initially, we prepared HAp and polysaccharide self-organized bodies with a precipitation method. The starting substances of the polysaccharides are used as chondroitin sulfate (ChS) or hyaluronic acid (HyA). The both polysaccharides were dispersed in the calcium hydroxide suspension. The phosphate solutions were added slowly into the calcium hydroxide suspensions with polysaccharides, adjusting the pH value of 8.00 at the room temperature. The nanostructure of HAp and polysaccharides self-organized bodies were observed with transmittance electron microscopy and were characterized with Fourier-transformed infrared spectroscopy (FT-IR) and thermogravimetry (TG).

Furthermore, we conducted the hybrid of collagen with the hydroxyapatite and polysaccharides self-organized bodies with a coprecipitation method. The collagens are type I or type II collagen (I-Col and II-Col) that were dispersed into phosphate solutions. The temperature and pH at the precipitation was controlled at 40 degree and 9.00.

To fabricate the porous materials of HAp/ChS/I-Col, HAp/ChS/II-Col, HAp/HyA/I-Col and HAp/HyA/II-Col with the composition of 60:15:25, the suspensions obtained and cross-linking agencies were mixed at room temperatures and freeze dried. The microstructures of the sponges materials created were examined with scanning electron microscopy (SEM).

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In-vitro evaluation system using human chondrocyte was developed to accomplish biologic evaluation for the materials semiquantatively for a short period of time. Primary cultured chondrocytes obtained during total knee arthroplasty for patients with osteoarthritis were used for biologic evaluation of the nanocomposites.

Results and discussion

We synthesized nanocomposite materials of HAp and biopolymers. The composites of HAp nanocrystals and polysaccharides with carboxyl groups that widely found at the connective tissues with self-assembled structures were obtained. The TEM image of the HAp / hyaluronic acid (polysaccharide) nanocomposite is shown in Fig. 1; the corresponding diffraction patterns taken over the whole regions are given as inlet. As seen in the electron diffraction pattern, the diffraction of 002 and 004 formed clear spots along the longitudinal axis of the corresponding island-like aggregation indicated by an arrow; therefore, the *c*-axes of the constituent HAp nanocrystals were aligned in parallel with the longitudinal axis of the aggregation. The self-assembled HAp crystals on biopolymers with carboxyl groups could be explained as an epitaxial growth.

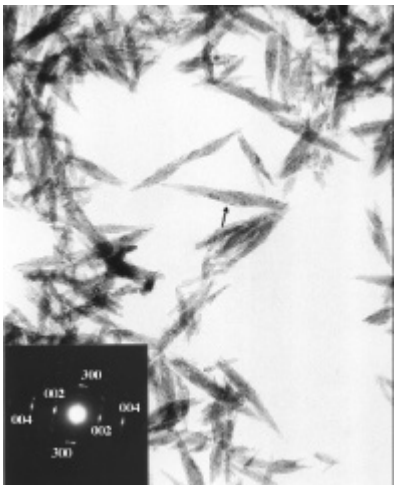


Fig. 1 TEM image of the nanocomposite of HAp and hyaluronic acid.

Fig. 2 shows one example of porous material that was fabricated like sheet. The size of the sheet was 25×15cm with the thickness of 1mm. The shape of the porous materials was easily fabricated. Fig. 3 shows the SEM images of microstructure of the nanocomposites. The averaged pore diameters were calculated to be 200-300μm.

Vigorous proliferation of the chondrocytes was observed in the composites. The rate of cell proliferation was different among the composites of different materials of hyarulonan and chondroitin sulfate, and collagen type I and type II. Cell culture test is a simple, biologic method and available for the evaluation of cell growth ratio and the production of cartilage matrix.

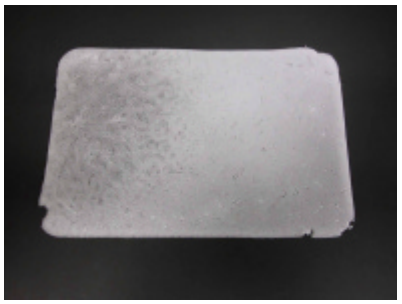
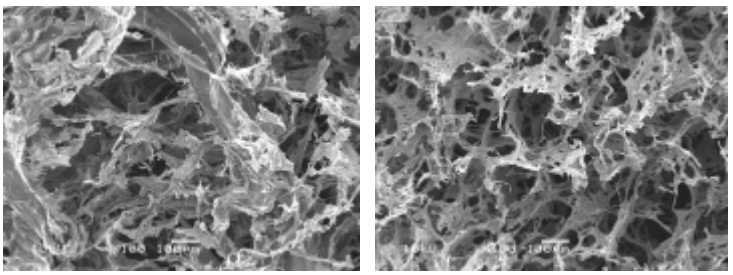


Fig.2 Macroscopic appearance.



HAp/HyA/I-Col sponge HAp/ChS/I-Col sponge

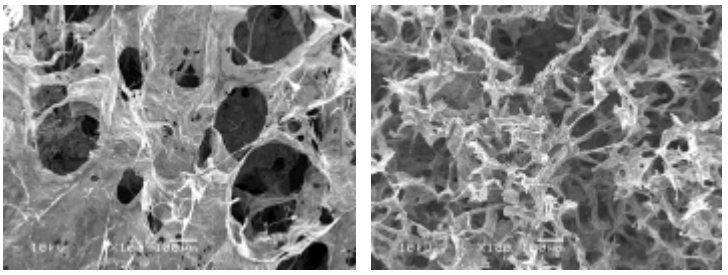


Fig. 3 SEM images of porous materials of nanocomposites.

HAp/HyA/II-Col sponge HAp/ChS/II-Col sponge